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Poly(2-propyl-2-oxazoline)s in Aqueous Methanol: To Dissolve or not to Dissolve

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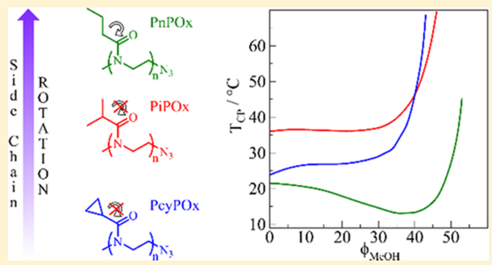
S *Supporting Information*

ABSTRACT: At room temperature, poly(*N*-isopropylacrylamide) (PNIPAM) is soluble in water and methanol, but it is not soluble in certain water/methanol mixtures. This phenomenon, known as cononsolvency, has been explored in great detail experimentally and theoretically in an attempt to understand the complex interactions occurring in the ternary PNIPAM/water/co-nonsolvent system. Yet little is known about the effects of the polymer structure on cononsolvency. To address this point, we investigated the temperature-dependent solution properties in water, methanol, and mixtures of the two solvents of poly(2-cyclopropyl-2-oxazoline) (PcyPOx) and two structural isomers of PNIPAM (M_n ∼ 11 kg/mol): poly(2-isopropyl-2-oxazoline) (PiPOx) and poly(2-*n*-propyl-2-oxazoline) (PnPOx). The phase diagram of the ternary water/methanol/poly(2-propyl-2-oxazolines) (PPOx) systems, constructed based on cloud point (T_{CP}) measurements, revealed that PnPOx exhibits cononsolvency in water/methanol mixtures. In contrast, methanol acts as a cosolvent for PiPOx and PcyPOx in water. The enthalpy, ΔH, and temperature, T_{max}, of the coil-to-globule transition of the three polymers in various water/methanol mixtures were measured by high-sensitivity differential scanning calorimetry. T_{max} follows the same trends as T_{CP}, confirming the cononsolvency of PnPOx and the cosolvency of PiPOx and PcyPOx. ΔH decreases linearly as a function of the methanol content for all PPOx systems. Ancillary high-resolution ^{1}H NMR spectroscopy studies of PPOx solutions in D_2O and methanol-d_4, coupled with DOSY and NOESY experiments revealed that the *n*-propyl group of PnPOx rotates freely in D_2O, whereas the rotation of the isopropyl and cyclopropyl groups of PiPOx and PcyPOx, respectively, is limited due to steric restriction. This factor appears to play an important role in the case of the PPOxs/water/methanol ternary system.



■ INTRODUCTION

At room temperature, poly(*N*-isopropylacrylamide) (PNIPAM) dissolves readily in water as well as in alcohols. Curiously, when methanol is added as a cosolute to an aqueous solution of PNIPAM, up to a molar fraction of ∼35 mol %, the mixed solution instantaneously turns turbid although water and methanol are miscible.[1,2] This phenomenon, called cononsolvency, is a consequence of the peculiar interactions of water molecules with PNIPAM in cold water, and bears similarity with the heat-induced dehydration and coil-globule collapse of PNIPAM chains that takes place in aqueous PNIPAM solutions above a temperature (T_{trans}) of around 32 °C.[3,4]

Polymer physicists and theoreticians still debate, sometimes argumentatively, the molecular origin of the loss of the PNIPAM solubility in certain water/methanol mixtures.[5−13] Over the years, various models, simulations, and theories were proposed. Okada and Tanaka extended to PNIPAM, the concept of cooperative hydration originally developed to explain the solubility of poly(ethylene glycol) in water.[14] Their model speculates that the formation of an amide−water hydrogen bond on one repeat unit of a dehydrated PNIPAM chain facilitates the hydration of the adjacent repeat unit, and the extension of hydration to longer sequences. The cooperativity of hydration accounts for the very fast collapse of the PNIPAM chain at the temperature where the water-amide H-bonds are broken. Tanaka et al. used the concept of hydration cooperativity in their model of the PNIPAM cononsolvency in water/methanol mixtures with the added assumption that methanol molecules interfere with the cooperative hydration of the amide units on the polymer. As a result of the competition, bare sections of repeat units form along the chains and the coiled chain collapses into a globule.[15] Pica et al. considered the geometric frustration experienced by solvent molecules to explain the cononsolvency of PNIPAM in water/methanol mixtures.[6] They argue that due to the presence of methanol, a significant number of binding sites along the polymer chain are inaccessible and remain unsolvated, leading to the collapse of the chain. In contrast, on the basis of molecular dynamics simulations, Mukherji et al. concluded that cononsolvency is driven by strong interactions of methanol with the polymer. Recently, van der Vegt et al.[7,8]

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considered the thermodynamics underlying the PNIPAM/water/methanol cononsolvency and proposed a model based on the entropy gain of the globular PNIPAM conformation in methanol/water solutions compared to solutions of PNIPAM in water.

Most of the theories, molecular dynamics simulations, and models developed over the years focus on the PNIPAM/water/methanol system and do not consider their extension to other polymers. It would be useful to understand the correlation between a specific structural motif and the occurrence of cononsolvency. Consider Figure 1, where we

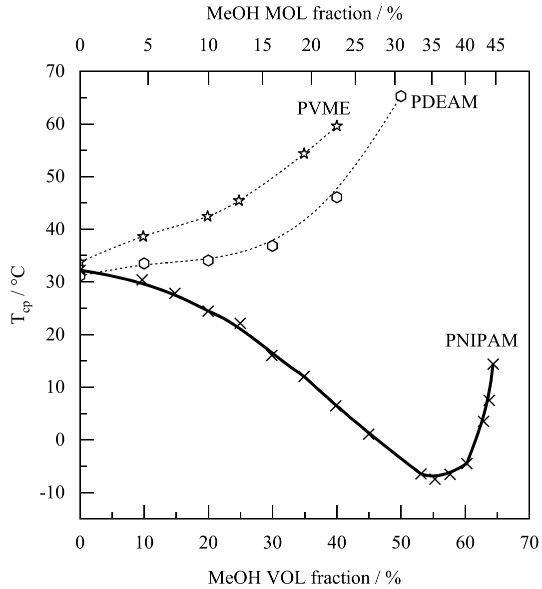
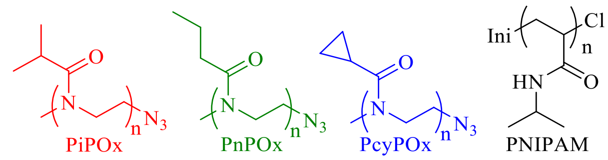


Figure 1. Reproduced phase diagrams of PVME,[2] PDEAM,[16] and PNIPAM[1] in water/methanol mixture.

present the phase diagrams of PNIPAM, poly(vinyl methyl ether) (PVME), and poly(diethyl acrylamide) (PDEAM) in water/methanol mixtures. Like PNIPAM, PVME and PDEAM are water-soluble polymers that exhibit a temperature-dependent coil-globule transition in water. Both polymers are soluble in methanol. Yet, the addition of methanol to an aqueous solution of PVME or PDEAM does not lead to cononsolvency. It results in the opposite effect: the temperature range in which the polymer is soluble increases.

To minimize the effects of end groups and molar mass on the coil-to-globule collapse of polymers in general[17] and on PNIPAM specifically,[5,18] we selected three poly(2-propyl-2-oxazoline)s (PPOx)s of similar molar mass and identical end groups. PPOxs consist of a $-(CH_2-N-CH_2)-$ main chain where the nitrogen atom is part of a tertiary amide function. A propyl group (*n*-propyl, isopropyl, or cyclopropyl) is linked to the carbonyl group of the amide (Scheme 1). On the basis of ^{1}H NMR spectroscopy analyses, we determined the preferred conformation of each PPOx in methanol-d_4 and D_2O below the phase transition temperature. Then, we built the phase diagrams of the three PPOxs in water/methanol mixtures via turbidimetry measurements and determined the enthalpy and phase transition temperature of the three polymers in mixed water−methanol solutions via microcalorimetry. The same measurements were carried out also with a PNIPAM sample of similar molar mass. It turned out that the three PPOxs had distinct phase diagrams in water/methanol mixtures. Careful

Scheme 1. Chemical Structure of the Polymers Investigated[a]



[a]The initiating group in PNIPAM is ethyl propionate.

analysis of high-resolution ^{1}H NMR spectra led us to pin-point the structural parameter responsible for cononsolvency within this limited set of polymers. This approach, which uses instrumentation commonly available in chemical laboratories, can be extended to other sets of polymers.

■ EXPERIMENTAL PART

Materials. The PPOxs were prepared following standard procedures,[19] as described in detail in the Supporting Information document. Their chemical structure is shown in Scheme 1. They carry a methyl group on one chain end and an azide on the other. The PNIPAM sample used as a control was prepared by atom transfer radical polymerization as described previously.[20] The molecular properties, glass transition temperatures (T_g), cloud points (T_{CP}) and surface tensions (γ_{H_2O}) in water of the polymers are listed in Table 1.

Table 1. Molecular Properties of the Polymers Investigated

#	polymer	M_{theo}[a]	M_n[b,c]	PD[b]	T_g[d]	T_{CP,H_2O}[d]	γ_{H_2O}[e]
1	PiPOx	11.5	26.1	1.14	66.9	36.0	49.5
2	PnPOx	11.2	27.7	1.22	29.0	21.3	42.3
3	PcyPOx	13.0	22.2	1.29	72.6	23.8	53.9
4	PNIPAM[f]	10.4	17.5	1.28		33.9	47.0

[a]Theoretical molecular weight in kg/mol according to $M_{monomer} \times Xp \times [M]/[I]$. [b]Obtained from size exclusion chromatography (SEC) (dimethylformamide (DMF), poly(methyl methacrylate) (PMMA) calibration). [c]In kg/mol. [d]In °C. [e]In mN/m, surface tension in water at 16° and a polymer concentration of 10 mg/L. [f]Data reproduced from ref 20.

Characterization. *NMR.* ^{1}H and ^{13}C NMR spectra of the polymer solutions (2.5 wt %) in D_2O and methanol-d_4 were recorded with a Bruker Avance III 500 MHz spectrometer and calibrated against the residual proton signal of the solvent. Standard Bruker pulse sequences as published in the Bruker pulse program catalog were used (zg30, zgpg30, ledbpgp2s, and noesyphsw). For diffusion ordered NMR spectra (DOSY) of the polymers in D_2O and methanol-d_4 (10 °C), the gradient strength was increased linearly 32 times while keeping the diffusion delay (d20) constant at 100 ms. Topspin 3.0 software was used to analyze the spectra. The obtained diffusion coefficients were converted to the hydrodynamic radius according to the Stokes−Einstein equation (η_{D_2O}: 1.679 mPas, η_{MeOD}: 0.788 mPas).[21] Two-dimensional (2D) Nuclear Overhauser Effect spectra (NOESY) were obtained at 10 °C and 2.5 wt % in D_2O and methanol-d_4.

SEC. Elugrams of the polymers (concentration: 2 g/L) eluted with DMF + LiBr (1 g/L) were obtained with a system consisting of a Waters 515 HPLC pump, a Biotech Model 2003 degasser, a Waters 717 plus autosampler, a guard column, and a Waters 2410 differential refractometer. The polymers were separated at an eluent flow rate of 0.8 mL/min with a set of Waters Styragel HR 2, 4, 6, 7, and 8 (x 300 mm) columns. The elugrams were analyzed with OmniSec software and calibrated against PMMA standards (Polymer Standard Service).

Fourier Transform Infrared (FTIR). Spectra of the polymers were recorded with a Bruker FTIR spectrometer α P at a resolution of 2 cm^{-1}.

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Preparation of Polymer Solutions in Water/Methanol Mixtures. Polymer stock solutions of identical polymer concentrations in water and methanol were prepared by placing weighed amounts of polymer and solvent in a vial, which was shaken vigorously until the dissolution of the solids, and stored at 4 °C for 16 h. To prepare the water/methanol mixtures (4 mL), the methanol stock solution was added to the aqueous stock solution under gravimetric control. The mixtures were shaken on a vortex mixer and kept at 4 °C for at least 2 h prior to measurements.

Construction of the PPOx Phase Diagrams in Mixed Water/Methanol Solutions. For PnPOx, PcyPOx and PiPOx, the phase diagrams were obtained with solutions having a polymer concentration of 10 g/L. The T_{CP} values used in the phase diagrams were obtained by transmittance measurements, as follows. Changes of the sample transmittance at $\lambda = 400$ nm with increasing temperature (1 °C/min) were observed with a UV/vis spectrometer V-750 (Jasco). A CTU-100 circulation thermostat unit coupled to an ETCR-762 Peltier cell holder was used to control and monitor the temperature of the polymer solutions (±0.1 °C) inside a quartz cuvette with a path length of 1 cm. The samples were equilibrated at the starting temperature for 10 min. The cloud point temperature was determined as the inflection point of the transmittance vs the temperature curve.

Differential Scanning Calorimetry (μDSC). Thermograms of the polymers in mixed solvents were recorded with a Malvern MicroCal PEAQ-DSC operating without active cell−cell compensation ("no-feedback mode"). The sample cell volume was 130 μL and the scan rate was set at 1 °C/min. To establish a reproducible thermal history of the instrument, the following protocol was implemented. First, a water/methanol mixture of composition identical to that of the sample to be evaluated was added manually in both the reference cell and the sample cell. The adiabatic jacket was pressurized (∼60 psi). The instrument was equilibrated for 5 min at 2 °C followed by a heating/cooling scan with 70 °C as the upper temperature. During the cooling scan, when the monitored temperature reached a value below the anticipated transition temperature of the polymer solution examined next, the adiabatic jacket was depressurized and the water/methanol mixture in the sample cell was replaced by the actual sample. The adiabatic jacket was pressurized, the cooling scan was completed. The system was equilibrated at 2 °C for 5 min. The polymer phase transition was recorded in the following heating scan. The baseline was recorded for each water/methanol mixture under identical conditions, except that the cells were not opened during the cooling scan. The baseline was subtracted using MicroCal PEAQ-DSC software. The data were normalized to the cell volume and concentration of repeating units. The area under the peak was integrated with the Origin software to give the transition enthalpy.

■ RESULTS

Solution Properties of PiPOx, PnPOx, and PcyPOx in Cold Water and Cold Methanol. The properties of the PPOxs were characterized by NMR spectral analysis of solutions of the PPOxs in either D_2O or methanol-d_4 kept at 10 °C, a temperature well below the T_{trans} of their solutions in water. We carried out DOSY experiments to gather information on the coil dimensions (Figures S3−S10) and NOESY experiments (Figures S12−S23) that correlate protons in close proximity through-space (<5 Å), to determine the three-dimensional (3D) arrangement of the polymer coils in solution. High-resolution ^{1}H NMR spectra were recorded as well (Figure 2) to identify slow rotating bonds.

In Table 2, we list the diffusion constants (D_{D_2O} and D_{CD3OD}) of the polymers obtained from DOSY spectra of polymer solutions (2.5 wt %) in D_2O and methanol-d_4. The hydrodynamic radii (R_h) of the polymers, obtained from the diffusion constants are given as well. The hydrodynamic radii of the polymers in D_2O (R_{h,D_2O}) are ∼2.5 times larger than the corresponding values in methanol-d_4 ($R_{h,CD3OD}$), which is

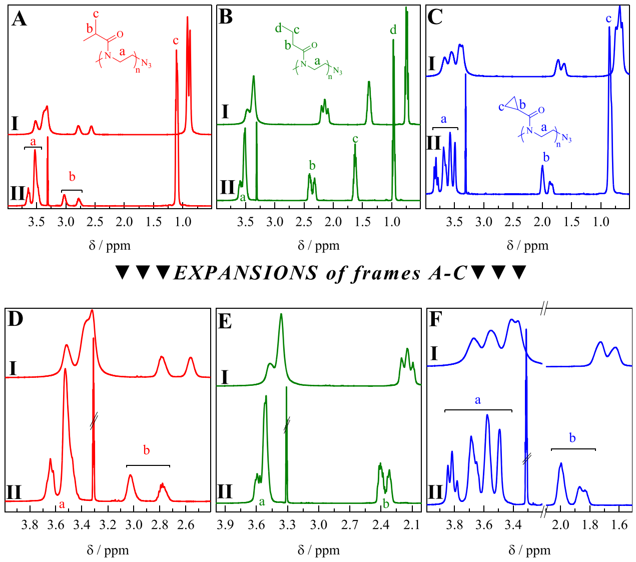

Figure 2. High-resolution ^{1}H NMR spectra in D_2O (I), and methanol-d_4 (II) of PiPOx (A, D), PnPOx (B, E), and PcyPOx (C, F). All spectra are normalized to the area under peak a. (D−F) Expansions of the spectral region exhibiting resonances (a) and (b).

equivalent to a 15-fold increase in the hydrodynamic volume. We take this as an indication that in water the polymer chains assemble in clusters of 10−15 chains. DOSY experiments were performed with dilute polymer solutions in D_2O (0.1 wt %). The recovered R_{h,D_2O} of the polymers in dilute solutions remained larger (∼4.5 nm) than the $R_{h,CD3OD}$ of the unimers measured in more concentrated solutions (2.5 wt %) (Figure S11).

Several strong off-diagonal cross-peaks were observed in 2D-NOESY spectra of PPOx solutions in D_2O. They are indicative of through-space inter-protons coupling over distances shorter than 5 Å. 2D-NOESY spectra of PPOxs in methanol-d_4 have fewer and weaker off-diagonal cross-peaks. These observations confirm that PPOxs exist as isolated chains in methanol-d_4 (Figures S12−S23), whereas in water they tend to aggregate even at temperatures well below T_{trans}.

Sections of the high-resolution ^{1}H NMR spectra of the PPOxs in D_2O (I, top spectra in each frame) and methanol-d_4 (II, bottom spectra in each frame) are shown in Figure 2. Overall, the spectra of the polymers in D_2O exhibit broader resonances than the spectra of the corresponding polymer solutions in methanol-d_4, which can be attributed to the more restricted motions of the aggregated chains in D_2O solutions, compared to methanol-d_4 (see above). The spectral domains presented in Figure 2 include the resonances of the main chain methylene protons (H_a) and the side-chain protons (H_b, H_c, and H_d, PnPOx only) (see structures within each frame of Figure 2). Note that the spectra in frames D−F of Figure 2 are expansions of the corresponding frames A−C. Particular attention should be paid to the resonances labeled a (−CH$_2$−N−CH$_2$−) and b (H_xC−CO, x: 1 or 2). These sets of signals are sensitive to the dihedral angles (−CH$_2$−N−C═O) and (H_x−C−C═O) (Scheme 2). If the rotation of the CO−N (α) or C−CO (ß) bonds is slow on the NMR time scale, the resonance of protons H_a and H_b are split according to the population of the different states imposed by the oxygen electron cloud.

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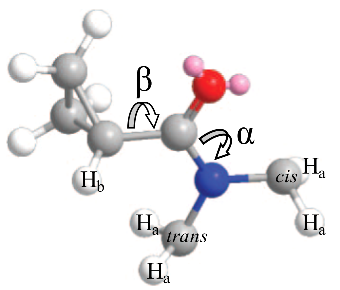
Table 2. Spectral Parameters Extracted from DOSY and High-Resolution NMR Spectra (Polymer Concentration: 2.5 wt %)

#	polymer	D_{D_2O}[a]	D_{CD3OD}[a]	R_{h,D_2O}[b]	$R_{h,CD3OD}$[b]	r_{D_2O}[c]	r_{CD3OD}[c]
1	PiPOx	1.7	9.5	7.3	2.8	0.55:0.5	0.75:0.5
2	PnPOx	1.9	9.8	6.5	2.7	uniform	0.68:0.5
3	PcyPOx	1.7	9.2	7.3	2.9	0.66:0.5	0.80:0.5
4	PNIPAM	2.1	10.4	5.9	2.5		

[a]Diffusion constants in 10^{-11} m^2/s. [b]Hydrodynamic radii in nm. [c]Ratio on the intensity (area) of the low-field to high-field signals due to the proton(s) H_b, α to the amide carbonyl of the repeat units (see Figure 2).

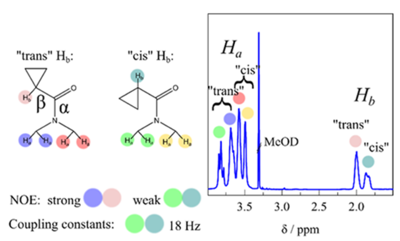
Scheme 2. Three-Dimensional Representation of One PcyPOx Repeating Unit[a]



[a]The slow rotating bonds CO−N (α) and C−CO (β) and the protons H_a and H_b are highlighted.

In the case of PcyPOx in methanol-d_4 (Figure 2, frames C and F, bottom spectra) the resonance of the methylene main chain protons (H_a) is split into four signals (3.4−3.9 ppm). The resonance of the methine proton (H_b) is split into two signals (1.8−2.1 ppm). Scheme 3 illustrates the assignments of the resonances to the protons of two specific conformers of PcyPOx. The two H_b resonances are split into the ratio r_{CD3OD} of 0.8:0.5 (Table 2). In the predominant conformation, the proton H_b is located "trans" to the oxygen, due to the steric constraint imposed by the cyclopropyl moiety. Similarly, the methylene groups (H_a) are split into two equal populations ("cis" and "trans" to the carboxyl oxygen).[22] The magnetic environments of two methylene groups are further influenced by the orientation of the methine proton, hence they have four distinct resonances in the ratio 1:1.6:1.6:1. On the one hand, the orientation of the electron-rich cyclopropyl moiety affects the electron cloud of the oxygen atom and therefore the two "cis" methylene protons (highlighted yellow and red in Scheme 3) experience different shieldings. On the other hand, the cyclopropyl protons and the methylene protons in the "trans" position (green and blue) are coupled through space. Both resonances of the methine proton exhibit cross-peaks with the "trans" methylene resonances in a 2D-NOESY spectrum due to the nuclear Overhauser effect. The "cis" methine signal and the "trans" methylene signal most downfield share the same coupling constant (18 Hz).

Scheme 3. Assignment of the ^{1}H NMR Resonances of PcyPOx in Methanol-d_4 to the "Trans" and "Cis" H_b Conformers[a]



[a]"Trans" and "cis" of the H_a methylene groups refer to their orientation toward the oxygen.

The ß-bond rotation of PiPOx and PnPOx in methanol-d_4 is slow as well, resulting in the splitting of resonances H_b indicative of the existence of two conformer populations in equilibrium (Table 2). The resonances H_a of PiPOx and PnPOx do not show the same splitting pattern as those of PcyPOx, an indication of the lesser steric demand of the n-propyl and isopropyl groups, compared to the cyclopropyl group. The side-group substituents in PiPOx and PnPOx are also less electron-rich than PcyPOx. The nature of the solvent also affects the ß-bond rotation and the equilibrium conformer population (Figure 2 and Table 2). The ratio of the peak areas of resonances H_b is more uniform in D_2O than in methanol-d_4. The spectrum of PnPOx in D_2O presents a single resonance H_b J-coupled to the adjacent $-CH_2-$. Hence, in D_2O, the n-propyl group exerts no detectable steric effect on bond rotation, on the NMR time scale.

In conclusion, the NMR studies give strong evidence that PPOxs exist as unimers in methanol and adopt a random coil conformation. The rotation of the side-chain amide substituent is limited only by intrachain steric effects. In contrast, PPOx chains tend to cluster in water in the form of small aggregates, an indication of their amphiphilicity revealed also by their surface tensions (see Table 1).

Temperature/Composition Dependence of Poly(2-propyl-2-oxazoline)s in Water/Methanol Mixtures. PiPOx, PnPOx, and PcyPOx are soluble in methanol at all temperatures up to the boiling point of methanol, whereas in water they exhibit a lower critical solution temperature. Although the polymers carry the same end groups and have approximately the same molar mass (M_n ∼ 11 kg/mol), their cloud points (T_{CP}) in water cover a wide temperature range from 21.5 °C (PnPOx) to 36.0 °C for PiPOx (see Table 1). The phase diagrams of PPOxs in water/methanol mixtures of variable composition are presented in Figure 3, together with the phase diagram of PNIPAM (M_{theo}: 10.4 kg/mol).[20] The phase diagrams of the three PPOxs present distinct features, with similarities between PiPOx and PcyPOx on the one hand, and PnPOx and PNIPAM on the other. The T_{CP}s of PiPOx and PcyPOx increase slightly upon addition of small amounts of methanol (<10% MeOH v/v). They remain nearly constant with increasing methanol concentration up to 20% (v/v). Further addition of methanol to the PiPOx solution leads to a gradual increase of T_{CP} until the methanol content reaches ∼38% (v/v); beyond this solvent composition, the T_{CP} value increases sharply and vanishes when the methanol content exceeds 46% (v/v). Qualitatively, the phase diagram of PcyPOx is similar to that of PiPOx. It differs slightly within the low MeOH content window of the phase diagram, for

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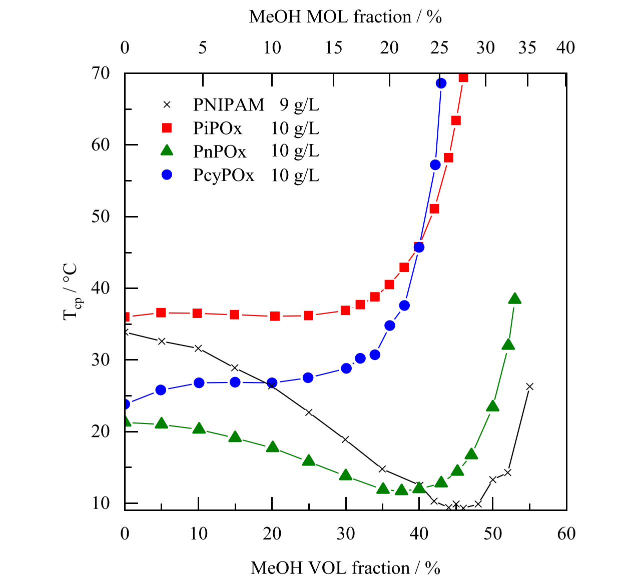

Figure 3. Cloud points of the different polymers in water/methanol mixtures as a function of MeOH volume fraction (bottom axis) and MeOH mol fraction (top axis).

which the T_{CP} increases by ~3 °C, from its value in water (23.8 °C) to a value of 26.8 °C for the water/methanol mixture containing 10% (v/v) methanol. The sharp increase of the PcyPOx solution T_{CP} starts for a methanol content of 36% (v/v). T_{CP} values increase rapidly up to 69 °C for a methanol content of 43% (v/v) and disappear. In the phase diagrams of PcyPOx and PiPOx, the T_{CP} values in mixed H_2O/MeOH solutions are never lower than their respective values in water. They remain constant over a wide solvent composition range before increasing and vanishing for mixtures of a higher methanol content. This feature indicates a delicate balance of opposing contributions. It sets PcyPOx and PiPOx apart from polymers, such as PVME and PDEAM (Figure 1), for which T_{CP} values increase gradually for all H_2O/MeOH compositions.

The phase diagram of PnPOx exhibits a net decrease of T_{CP} upon an increase of the methanol content to reach the minimum value of ~12 °C in solutions containing 35% (v/v) MeOH. Further increase of the methanol content leads to an increase of T_{CP}s until the methanol content reaches 53% (v/v). Mixed solutions of higher methanol content remained clear at all temperatures. The phase diagram of PnPOx is similar to that of PNIPAM (see Figure 3), although the amplitude of the drop of T_{CP} upon increasing the MeOH content is smaller (9.6 vs 23.9 °C in the case of PNIPAM) and the minimum is shallower. Nonetheless, it has the features characteristic of cononsolvency reported first in the case of PNIPAM.[1,2]

Thermograms of the PPOx Samples in Water/Methanol Mixtures. To trace the origin of the distinct characteristics of the PPOx/water/methanol mixtures and their changes with temperature, we carried out high-sensitivity differential scanning calorimetry (μDSC) measurements that provide the enthalpy associated with the coil-globule transition and the release of polymer-bound solvent to bulk solvent. The thermograms of PnPOx, PiPOx, PcyPOx, and PNIPAM in water/methanol mixtures of various methanol contents are presented in Figures 4 and Figure S25 (PNIPAM). The thermograms were obtained after subtraction of the reference thermogram recorded with a water/methanol composition identical to that of the corresponding sample. The topmost thermogram in each frame of Figure 4 corresponds to the polymer solution in water. The methanol content of the mixed samples increases from the top to the bottom thermogram (see arrow in Frame A). For the sake of clarity, the scale of the ordinate is different in each frame. The polymer concentration was set at 10 g/L to ensure that the heat transfer remains detectable for a methanol content as high as possible. In the case of the PnPOx solution, we used a lower concentration (5 g/L) as the enthalpy of 10 g/L PnPOx exceeded the detection limit of the μDSC detector. To confirm that the thermal properties of the two samples were identical, we measured the phase diagram at 5 g/L of PnPOx in aqueous methanol solution. It was nearly identical to that of the 10 g/L PnPOx aqueous solution (see Figure S24). The difference, ΔT_{CP}, between the two T_{CP} values is less than 1 °C for $\phi_{MeOH} < 37.5$ vol % and 1−2 °C for $37.5 < \phi_{MeOH} < 50$ vol %. In the 50−53 vol % MeOH composition range where the T_{CP} increase is very sharp, ΔT_{CP} tends to increase to ~4 °C, possibly due to larger experimental errors in this composition range.

Considering first the thermograms recorded for polymer solutions in water, we note that the transition enthalpy of the PnPOx solution is the largest (6.9 kJ/mol) of the four samples.

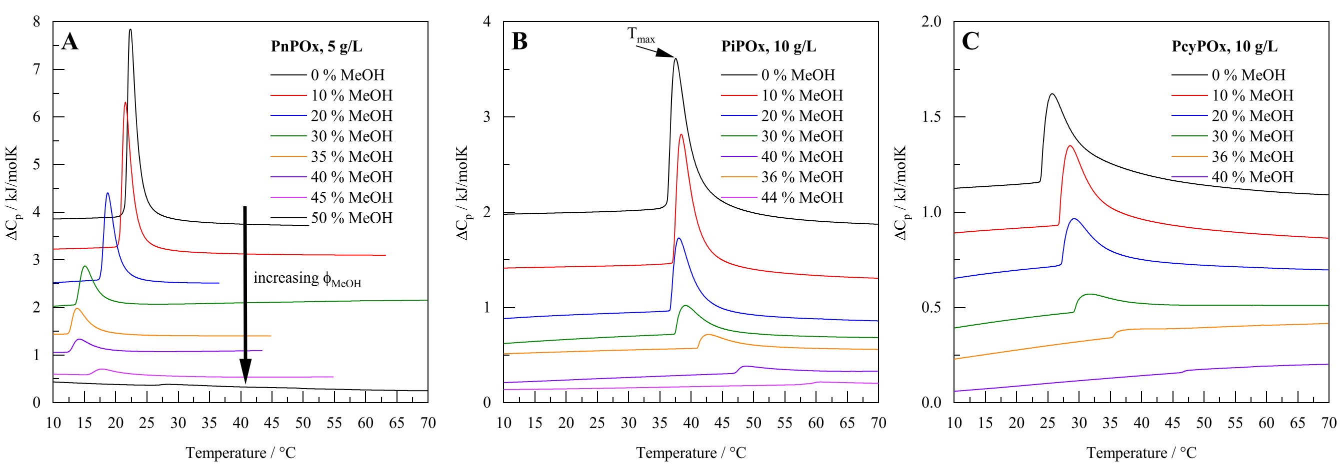

Figure 4. Thermograms of (A) PnPOx, (B) PiPOx, and (C) PcyPOx in aqueous methanol solutions. The methanol contents are given in % vol fraction. Note the different *y*-scales.

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ΔH values of the other polymers in water decrease in the following order: PNIPAM (6.2 kJ/mol), PiPOx (5.6 kJ/mol), and PcyPOx (3.2 kJ/mol). This order is the same as that of the T_g values of the dry polymers (Table 1). It also correlates with the relative freedom of rotation of the side groups of the three polymers in D_2O: the *n*-propyl group of PnPOx rotates freely, while the rotation of the isopropyl and cyclopropyl side groups of PiPOx and PcyPOx are restricted, especially in the case of PcyPOx. The shape of the endotherm, also, varies depending on the structure of the polymer side group. The phase transitions of PnPOx and PNIPAM (Figure S24) are characterized by sharp, symmetrical endotherms with a full width at half maximum (FWHM) of 1.5 °C (PnPOx) and 2.6 °C (PNIPAM) typical of cooperative transitions. The FWHM is significantly broader in the case of PcyPOx (5.1 °C) and, to a lesser extent, in the case of PiPOx (2.8 °C) and the transition endotherms exhibit a pronounced tailing on the high-temperature side. Both the increase of FWHM and the asymmetry of the endotherm are characteristics of non-cooperative transitions.[14]

The thermograms of mixed samples in Figure 4 yield information on the variations with solvent compositions of the temperature, T_{max}, and the enthalpy of the desolvation of the polymer chain during the phase separation. The T_{max} recorded for PnPOx (Figure 4A) mixed solutions decreases to lower temperatures with the increasing methanol content, reaches a minimum value (T_{max}: 13.8 °C), and increases with further increase in methanol content. Similar trends are observed in the thermograms recorded with PNIPAM mixed solutions (Figure S25). They are the fingerprint of cononsolvency. For PiPOx and PcyPOx mixed solutions, T_{max} increases gradually with the increasing methanol content (Figure 4), confirming that both PiPOx and PcyPOx exhibit cosolvency in mixed H_2O/methanol. In all cases, as the methanol content increases, the transition enthalpy decreases, as observed also by Schild et al.[2] in their comparative study of PNIPAM (cononsolvency) and PVME (cosolvency) in water/MeOH mixtures. In Figure 5, we plot ΔH of the three PPOx samples and PNIPAM as a function of the methanol volume fraction (see Table S2). The ΔH vs ϕ_{MeOH} decay is linear, with slopes of −0.13 kJ/mol for PNIPAM and PiPOx, −0.15 kJ/mol for PnPOx and −0.08 kJ/mol for PcyPOx.

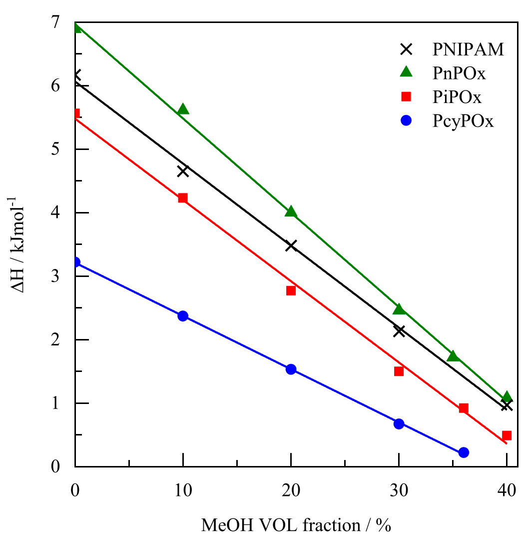

Figure 5. Changes of ΔH as a function of ϕ_{MeOH} for PPOxs and PNIPAM. The lines drawn through the data points are linear fits to the data.

In summary, the microcalorimetry studies lead us to conclude that in mixed water/methanol PnPOx, like PNIPAM exhibits cononsolvency and undergoes a cooperative transition at T_{max} over the $0 < \phi_{MeOH} < \sim 50$ vol % range. PiPOx and PcyPOx exhibit consolvency in mixed water/MeOH and their desolvation at T_{trans} is not cooperative. The ^{1}H NMR studies of PPOx solutions in water reported in the first part of the report indicate that the *n*-propyl side chains of PnPOx undergo free rotation, while the rotation of the cyclopropyl and isopropyl side chains of PcyPOx and PiPrOx, respectively, is restricted due to steric hindrance. Since the three polymers possess the same hydrogen-bond acceptor moiety, our results imply that the extent of rotational freedom is a controlling factor in determining the occurrence of cononsolvency in ternary PPOx/water/methanol systems.

In polymer solutions, the random coil (C) and the globular (G) chain conformations are in permanent exchange ($C \rightleftharpoons G$). The equilibrium constant ($K = [G]/[C]$) depends on the temperature and the presence of cosolutes. In the case of aqueous PNIPAM solutions, at temperatures below T_{trans}, the polymer forms hydrogen bonds with water molecules and adopts preferentially a coil conformation ($K < 1$).[8] At temperatures above T_{trans}, the chains dehydrate and collapse to form strongly scattering globules ($K > 1$). $K = 1$ for $T = T_{trans}$ and the Gibbs free energy ($\Delta G = \Delta H - T\Delta S$) is zero, or

$$T_{trans} = \Delta H/\Delta S \tag{1}$$

Equation 1 implies that T_{trans} varies if the ratio of ΔH over ΔS changes. In the case of ternary PPOx/water/methanol solutions of different composition, we have shown here that ΔH of the PPOx phase transition correlates well with the methanol content of the ternary system, but it does not correlate with changes in T_m values. This leads one to conclude that the entropy contributions take a central role in the occurrence of cononsolvency or cosolvency. The transition entropy consists of a contribution related to the extent of solvent molecules adsorption/release on/from the polymer (ΔS^{H_2O} and ΔS^{MeOH}, respectively) and a contribution due to the changes in configurational entropy ($\Delta S^G - \Delta S^C$)

$$\Delta S = \Delta S^{H2O} + \Delta S^{MeOH} + \Delta S^G - \Delta S^C \tag{2}$$

The ΔS^{H_2O} and ΔS^{MeOH} terms depend, in part, on the degree of binding of MeOH and water to the polymer chain and globule. In the case of PNIPAM, the presence of methanol in the collapsed globules leads to an increased ΔS^G contribution, which favors the decrease of T_{trans}, i.e., cononsolvency.[7,8] In a recent study of the macroscopic liquid−liquid phase separation (MLLPS) of PNIPAM in water/methanol mixtures, we determined experimentally the composition of the polymer-rich phase recovered. This phase contained both water and methanol, but it was depleted significantly in methanol, compared to the initial solution composition.[23] Preliminary results of the MLLPS of the PPOx in water/methanol mixtures point to a similar depletion of the methanol content in the polymer-rich phase.

■ CONCLUSIONS

The molecular origin of cononsolvency in aqueous media is still under debate. Previous studies investigating the cononsolvency of PNIPAM in water/methanol mixtures point to the central role of the solvent structure around the NH amide

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proton of PNIPAM. This point is mute in the case of poly(2-propyl-2-oxazoline)s, which contain solely ternary amide groups. The three PPOxs chosen here share some common solution properties: they are surface-active and form small aggregates in cold water, whereas in cold methanol they dissolve as unimers. They also exhibit considerable differences, especially in terms of the rotation freedom of the 2-propyl substituent. The thermodynamic parameters of the phase transition in water, namely, the transition temperature and transition enthalpy, differ drastically among the three PPOxs. PnPOx exhibits cononsolvency in a given composition range of methanol/water mixture solubility. This is not the case for either PiPOx or PcyPOx. A thermodynamic study of the phase transition of PPOxs in water/methanol mixtures indicates that the transition enthalpy decays linearly with the addition of methanol, irrespectively of the occurrence of cononsolvency. The degree of freedom of the polymer chain affects the entropy/enthalpy balance, and ultimately controls the macroscopic properties of PPOx in water/methanol mixtures. This work presents important new experimental insights into the solution properties of PPOxs that ought to be taken into consideration in theoretical predictions and molecular simulations.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.macromol.9b01234.

Experimental section, PPOx synthesis, and molecular characterization, DOSY spectra, concentration dependence of R_h of PcyPOx in D_2O and MeOD, NOESY spectra, concentration dependence of the PnPOx phase diagram, thermograms of PNIPAM and tabulated values extracted from μDSC (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Winnik, F. M.; Ringsdorf, H.; Venzmer, J. Methanol-Water as a Co-Nonsolvent System for Poly(N-Isopropylacrylamide). *Macromolecules* **1990**, *23*, 2415−2416.

(2) Schild, H. G.; Muthukumar, M.; Tirrell, D. A. Cononsolvency in Mixed Aqueous Solutions of Poly(N-Isopropylacrylamide). *Macromolecules* **1991**, *24*, 948−952.

(3) Heskins, M.; Guillet, J. E. Solution Properties of Poly(N-Isopropylacrylamide). *J. Macromol. Sci., Part A: Chem.* **1968**, *2*, 1441−1455.

(4) Halperin, A.; Kröger, M.; Winnik, F. M. Poly(N-Isopropylacrylamide) Phase Diagrams: Fifty Years of Research. *Angew. Chem., Int. Ed.* **2015**, *54*, 15342−15367.

(5) Tanaka, F.; Koga, T.; Kojima, H.; Xue, N.; Winnik, F. M. Preferential Adsorption and Co-Nonsolvency of Thermoresponsive Polymers in Mixed Solvents of Water/Methanol. *Macromolecules* **2011**, *44*, 2978−2989.

(6) Pica, A.; Graziano, G. An Alternative Explanation of the Cononsolvency of Poly(N-Isopropylacrylamide) in Water−Methanol Solutions. *Phys. Chem. Chem. Phys.* **2016**, *18*, 25601−25608.

(7) Dalgicdir, C.; Rodríguez-Ropero, F.; van der Vegt, N. F. A. Computational Calorimetry of PNIPAM Cononsolvency in Water/Methanol Mixtures. *J. Phys. Chem. B* **2017**, *121*, 7741−7748.

(8) Rodríguez-Ropero, F.; Hajari, T.; van der Vegt, N. F. A. Mechanism of Polymer Collapse in Miscible Good Solvents. *J. Phys. Chem. B* **2015**, *119*, 15780−15788.

(9) Mukherji, D.; Wagner, M.; Watson, M. D.; Winzen, S.; de Oliveira, T. E.; Marques, C. M.; Kremer, K. Relating Side Chain Organization of PNIPAm with Its Conformation in Aqueous Methanol. *Soft Matter* **2016**, *12*, 7995−8003.

(10) Mukherji, D.; Marques, C. M.; Kremer, K. Polymer Collapse in Miscible Good Solvents Is a Generic Phenomenon Driven by Preferential Adsorption. *Nat. Commun.* **2014**, *5*, No. 4882.

(11) Bischofberger, I.; Calzolari, D. C. E.; Trappe, V. Co-Nonsolvency of PNiPAM at the Transition between Solvation Mechanisms. *Soft Matter* **2014**, *10*, 8288−8295.

(12) Bischofberger, I.; Calzolari, D. C. E.; De Los Rios, P.; Jelezarov, I.; Trappe, V. Hydrophobic Hydration of Poly-N-Isopropyl Acrylamide: A Matter of the Mean Energetic State of Water. *Sci. Rep.* **2015**, *4*, No. 4377.

(13) Zhang, G.; Wu, C. The Water/Methanol Complexation Induced Reentrant Coil-to-Globule-to-Coil Transition of Individual Homopolymer Chains in Extremely Dilute Solution. *J. Am. Chem. Soc.* **2001**, *123*, 1376−1380.

(14) Okada, Y.; Tanaka, F. Cooperative Hydration, Chain Collapse, and Flat LCST Behavior in Aqueous Poly(N-Isopropylacrylamide) Solutions. *Macromolecules* **2005**, *38*, 4465−4471.

(15) Tanaka, F.; Koga, T.; Winnik, F. M. Temperature-Responsive Polymers in Mixed Solvents: Competitive Hydrogen Bonds Cause Cononsolvency. *Phys. Rev. Lett.* **2008**, *101*, No. 028302.

(16) Maeda, Y.; Yamabe, M. A Unique Phase Behavior of Random Copolymer of N-Isopropylacrylamide and N,N-Diethylacrylamide in Water. *Polymer* **2009**, *50*, 519−523.

(17) de Gennes, P.-G. *Scaling Concepts in Polymer Physics*; Cornell University Press: Ithaca and London, 1979.

(18) Qiu, X.; Koga, T.; Tanaka, F.; Winnik, F. M. New Insights into the Effects of Molecular Weight and End Group on the Temperature-Induced Phase Transition of Poly(N-Isopropylacrylamide) in Water. *Sci. China:Sci. China: Chem.Sci. China: Chem. Chem.* **2013**, *56*, 56−64.

(19) Bloksma, M. M.; Weber, C.; Perevyazko, I. Y.; Kuse, A.; Baumgärtel, A.; Vollrath, A.; Hoogenboom, R.; Schubert, U. S. Poly(2-Cyclopropyl-2-Oxazoline): From Rate Acceleration by Cyclopropyl to Thermoresponsive Properties. *Macromolecules* **2011**, *44*, 4057−4064.

(20) Karjalainen, E.; Aseyev, V.; Tenhu, H. Upper or Lower Critical Solution Temperature, or Both? Studies on Cationic Copolymers of N-Isopropylacrylamide. *Polym. Chem.* **2015**, *6*, 3074−3082.

(21) Evans, R.; Deng, Z.; Rogerson, A. K.; McLachlan, A. S.; Richards, J. J.; Nilsson, M.; Morris, G. A. Quantitative Interpretation

of Diffusion-Ordered NMR Spectra: Can We Rationalize Small Molecule Diffusion Coefficients? *Angew. Chem., Int. Ed.* **2013**, *52*, 3199−3202.

(22) Kowalewski, V. J.; de Kowalewski, D. G. Proton NMR Spectra of the N,N-Dimethylformamide, N-Methylformamide, and N,N-Dimethylacetamide. *J. Chem. Phys.* **1960**, *32*, 1272−1273.

(23) Xue, N.; Qiu, X.-P.; Aseyev, V.; Winnik, F. M. Nonequilibrium Liquid−Liquid Phase Separation of Poly(N-Isopropylacrylamide) in Water/Methanol Mixtures. *Macromolecules* **2017**, *50*, 4446−4453.

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